The fast, low affinity, Ca^{2+} dye Mag-Fluo-4 loaded in its AM moiety, in mouse FDB fast-twitch fibers has allowed to study, with high temporal resolution, the decay phase of single or tetanic (350 ms-100 Hz), Ca^{2+} transients (Calderón et al. JMRCM 2009;30). At 23°C, a double exponential function with time constants (always expressed in ms), τ_1=1.4 ± 0.1, τ_2=15.3 ± 1.3 (n=20), and τ_1=23.4 ± 2.5, τ_2=594 ± 102.4 (n=13), respectively, describe the decay phase of single and tetanic transients. These time constants have been associated with Ca^{2+} binding to parvalbumin, and SERCA, respectively. We tested for differential effect of tetanic Ca^{2+} loads and temperature on the putative Ca^{2+} removal mechanisms. Increasing temperature, from 23 to 33°C, reversibly diminishes the single transient amplitude (ΔF/F), by 31%, and increases the base line by 32% (n=25). At 33°C, in 15 out of 20 fibers, the decay of single transients followed a single exponential, τ=4.7 ± 0.7, while in the other 5 fibers, two exponentials provided a better fit, τ_1=0.6 ± 0.1 and τ_2=5.1 ± 0.4; two exponentials τ_1=10.1 ± 1.8 and τ_2=394.9 ± 51.2 (n=13), provided the best fit for tetanic decay. The results show that the two time constants, that describe Ca^{2+} single and tetanic responses decay, are differentially affected by temperature and by tetani imposed Ca^{2+} loads. The fast decay components of single and tetanic responses, represented by τ_1, and associated with parvalbumin Ca^{2+} binding, are affected similarly by Ca^{2+} load (17x increase) or by temperature (2.3x increase for 10°C increase). The slower components of the single and tetanic responses, represented by τ_2, and associated with SERCA operation, are differentially affected by a 10°C temperature raise (3.1x vs 1.5x) and by the tetanic Ca^{2+} load (39x vs 77x) respectively.

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Cellular Mechanisms of Cardiac Depression and Recovery in Endotoxemic Mice

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Sepsis and septic shock are associated with a reversible cardiac dysfunction, that complicates management and worsens prognosis. In patients that survive septic shock, cardiac function recovers spontaneously, through mechanisms that are currently unknown. Here we aimed to identify the intracellular calcium (Ca^{2+}) transporters responsible for cardiac recovery after endotoxemic challenge in mice. Male C57Bl6 mice were administered lipopolysaccharide (LPS, 7 µg/g weight, ip). 12h after LPS administration, cardiomyocyte sarcomere shortening (SS) and Ca^{2+} transients (ΔCai, measured with fura2-AM) were decreased to 53 ± 10% and 78 ± 5% of control, respectively, in association with a decrease in trans-sarcolemmal Ca^{2+} influx (Cainf) through the L-type Ca^{2+} channels (LTCC, to 65 ± 9%) and sarcoplasmic reticulum (SR) Ca^{2+} pump (SERCA) reuptake (88 ± 5%, n > 19 cells from 3 mice for all). SR Ca^{2+} load (CaSR, measured with caffeine) was unchanged, while SR fractional release (FR, as αCai/CaSR) was decreased to 84 ± 6% of control (n's as above). 72h after LPS administration, survival was 40%. In cells isolated from surviving mice 72h after LPS, ΔCa, FR and Cainf were fully recovered, while SERCA showed a supranormal function (138 ± 4% of control, n's as above). SS showed a trend towards a partial depression (to 82 ± 7% of control, p = 0.077, n's as above) at 72h, that persisted up to 6 days after LPS administration. In conclusion, the recovery of cardiac Ca^{2+} handling after LPS challenge is associated with a full recovery of LTCC dysfunction, a supranormal activation of SERCA, despite, possibly, a persistent dysfunction in the contractile mechanisms downstream of the Ca^{2+} transient.

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Conditional Up-Regulation of SERCA2a Exacerbates Ventricular and Atrial Arrhythmias in the Setting of Catecholaminergic Polymorphic Ventricular Tachycardia

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SERCA2a gene transfer is an emerging therapy for treating contractile dysfunction in heart failure. While improving contractile performance, SERCA2a overexpression has been shown to exacerbate arrhythmias, although beneficial effects of SERCA2a up-regulation on cardiac rhythm have also been reported. To examine the role of SERCA2a and the consequences of its acute up-regulation in arrhythmogenesis, we conditionally overexpressed SERCA2a in a genetic mouse model featuring catecholaminergic polymorphic ventricular tachycardia (CPVT) due to loss of calsequestrin 2 (CASQ2). CASQ2 knock-out (KO) mice were crossbred with doxycycline (DOX)-inducible SERCA2a transgenic mice to generate KO-TG mice. In-vivo ECG studies showed that uninduced KO-TG (DOX-) mice developed both ventricular and atrial arrhythmias in response to catecholamine challenge (isoproterenol, ISO or a combination of ISO and caffeine). Induction of SERCA2a (DOX^+) markedly exacerbated both ventricular and atrial arrhythmias in response to ISO. Besides ventricular bigeminy, KO-TG (DOX^+) also displayed frequent bursts of sustained ventricular ectopic beats that were not present in KO-TG (DOX-). Moreover, episodes of atrial rhythm disturbances in KO-TG (DOX^+) mice occurred even under baseline conditions (no ISO). ISO further promoted atrial tachy- and brady-arrhythmias in the KO-TG (DOX^+) mice. Consistent with the in-vivo studies, confocal Ca imaging in both ventricular and atrial myocytes demonstrated that acute SERCA2a overexpression significantly increased the rate of occurrence of diastolic spontaneous and triggered Ca release events. Thus, our results suggest that acute overexpression of SERCA2a exacerbates both ventricular and atrial arrhythmias in settings of CPVT by further elevating diastolic Ca release.

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Fractal-Like Behavior of the Heart-Beat Intervals is Encoded within Intrinsic Complexity of Pacemaker Cells Residing in the Sinoatrial Node and Modulated by Autonomic Input to the Heart

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The heart rate and rhythm are controlled by complex chaotic neural, chemical and hormonal networks which are not strictly regular, but exhibit fluctuations across multiple-time scales. Therefore, it is not surprising that decoding of the ECG in mammals, even under resting conditions, reveals scale-invariant dynamics and beat-interval variability (BIV). Moreover, fractal-like behavior of heart beat intervals, which reveals itself in a power-law dependence of the frequency distribution of its intrinsic regimes, contributes to the complexity of the mammalian heart's rhythm. The traditional explanation for BIV and fractal-like behavior of heart beat intervals is that this result from the balance of sympathetic and parasympathetic autonomic input to the heart. But whether the sinoatrial node (SAN), the heart's primary pacemaker, or pacemaker cells isolated from the SAN exhibit fractal-like behavior of beating intervals in the absence of autonomic input is unknown.

We analyzed beating rhythms: (i) in vivo, when the brain input to the SAN is intact; ii) during autonomic denervation in vivo; iii) in intact isolated (denervated) SAN; and iv) in single pacemaker cells isolated from the SAN. By segregating each component of the brain-pacemaker cascade, we discovered that fractal-like beating interval exhibit in SAN tissue and although the beat interval of single pacemaker cells isolated from the SAN is rhythmic it does not exhibit fractal-like behavior. Therefore, cell-to-cell communication among pacemaker cells within the SAN tissue is required to impart their fractal-like beating interval behavior within the SAN. Autonomic input from the brain to the heart in vivo modulates both the rate and rhythm at which pacemaker cells beat and its fractal-like behavior, but it is not required for isolated SAN fractal-like complexity.

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The Mstn-Cmpt Dl1Abc- Mice. A Mouse Model to Study Muscle Weakness, Fatigue and Soce

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In our mouse model, a naturally occuring 12-bp deletion in the myostatin gene is considered responsible for the compact phenotype (Mstn(Cmpt-dl1Abc)) labeled by a tremendous increase in body weight along with signs of muscle weakness and easier fatigability.

Western blot screenings showed significantly reduced endogenous STIM1 and Orai1 protein levels in the compact mouse muscle samples. As a consequence, we hypothesized that SOCE may be consequently altered. Enzymatically isolated fluo-8 AM loaded FDB fibers from wild type and Mstn(Cmpt-dl1Abc) mice were used. To elicit a massive SR Ca^{2+}-release a RyR1 agonist (4-chloro-meta-cresol) was applied in a Ca^{2+} free medium and in the presence of the SR Ca^{2+} pump inhibitor (thapsigargin). The above cocktail triggered a